**AMENDMENTS TO THE SPECIFICATION:**

Applicants refer to paragraphs in the specification according to the paragraph numbers set forth in published application US 2004/0161414 A1. Applicants make these changes to correct grammatical and typographical errors, as requested by the Office. Please replace the current abstract with the attached abstract.

Also, please amend the specification as follows:

[0002] This invention relates to a novel method ~~to cure~~ of curing spinal cord injuries (SCI) and therapeutic agents for ~~that~~ curing SCI. In detail, this invention relates to ~~the~~ a method ~~to cure~~ of curing spinal cord injuries by local injection of CNS glial cells into injured sites of the spinal cord of SCI patients and to therapeutic agents whose active constituent is central nervous system (CNS) glial cells.

[0003] Spinal cord injuries induce serious symptoms: paraplegia (paralysis of lower extremities on both sides) or quadriplegia or even respiratory paralysis in addition to quadriplegia, and accordingly a wheelchair or a bedridden life is unavoidable. To date effective therapies to cure SCI have not been found yet. ~~Being deprived of the freedom of~~ In a short moment, an accident can render a person unable to use his or her hands and/or feet. ~~hand and foot by a momentary accident of traffic or sports the~~ These patients desperately hope to move ~~his or her~~ their own hands and feet and walk again by restoration of the damaged nerve pathways.

[0005] A hypothesis regarding CNS environment has newly arisen and permeated as a dogma. It claims that the environment of the mammalian CNS is non-permissive to axonal growth and consequently it is necessary to make the environment permissive to induce regeneration of axons. Schwab and his collaborators found myelin

associated growth inhibitory factor in the CNS white matter and supposed that the factor is responsible for the non-permissiveness of the mammalian CNS. In fact, they reported that neutralization of the factor by antibody induces regeneration of the pyramidal tract after transection and extension of the tract occurs across the lesion in adult rats [Nature, 343(18), 269(1990)]. Besides their reports, various attempts to make the CNS environment permissive were made to induce regeneration of the spinal cord in adult rats: transplantation of a peripheral nerve segment, Schwann cells, and olfactory ensheathing cells (OEC, glial cells specific to olfactory nerve and bulb). Cheng and his collaborators reported the occurrence of functional recovery after removal of spinal cord segments in adult rats by bridging a peripheral nerve segment in the gap of the vacated spinal cord [Science, 273 (26), 510 (1996)]. Guest and his collaborators reported the occurrence of regeneration of the spinal cord by grafting Schwann cells, that is, glial cells of the peripheral nervous system (PNS) [Exp. Neurol., 148, 502 (1997)]. Li and his collaborators reported the occurrence of regeneration of the pyramidal tract and functional recovery by grafting cultured OEC into the lesion after partial transection of the upper cervical cord [Science, 277(26), 2000 (1997)]. Regenerated projections by these attempts to make the environment permissive, however, were small in amount and short in length (at most 10 mm) and mostly aberrant, not reaching the proper targets. Consequently the extent of functional recovery was so small that hindlimbs could not fully support the body weight. To ~~let the~~ free SCI patients ~~free~~ from the wheelchair and allow them to walk again ~~by~~ on their own feet, ~~it is seriously wanted~~ there is a serious need to develop a novel curative method that makes it possible to reconstruct neural projections similar to normal projections.

[0007] This invention is based on our hypothesis that the local conditions in the lesion but not the global non-permissive environment of the CNS are responsible for the failure of axonal regeneration. The hypothesis is quite different from the currently held view but consistent with all findings in our spinal cord injury studies: we found that marked regeneration of transected CNS pathways occurs spontaneously after a sharp cut in rats younger than one month of age. In adult rats ranging from 2 to 3 months old spontaneous regeneration did not occur presumably because CNS tissue is harder compared with young animals and thus transection induces edema in the lesion. However, when grafted with embryonic rat spinal cord tissue into the lesion axonal regeneration similar to normal projections was induced. Therefore, we presumed that the CNS environment is not globally non-permissive and failure of regeneration is due to deterioration of the local conditions, i.e., perturbation of axon guidance cues that enable growing axons to find correct path and targets. It appeared very likely that various attempts to make the CNS environment permissive break consistency of axon guidance cues and consequently restrict regeneration of axons in amount and extension resulting in aberrant projections. On the basis of our hypothesis, we attempted to ameliorate the local conditions in the lesion by grafting cultured mixed glial cells harvested from neonatal rat spinal cord, expecting restoration of the microenvironment in the lesion. The glial cells were grafted into the lesion after a complete transection of the spinal cord at ~~a~~ the level of thoracic segments in adult rats. The grafted animals recovered from complete paraplegia to walking nearly normally. Regenerated projections were similar to normal in amount, extension, path, and termination. This invention was achieved by a novel insight into regeneration of the mammalian CNS. ~~Diametrically opposite~~

Contrary to the conventional concept that CNS glial cells impede axonal regeneration, we succeeded in achieving neural repair of the spinal cord using such cells. The extent of restoration of neural connections and recovery of function were much higher than those in the attempts performed on the basis of the conventional concept.

[0008] This invention offers a method of curing spinal cord injury by transplantation of CNS glial cells into the injured spinal cord in humans or other mammals. The cells for transplantation comprise at least ~~a~~ one kind of cultured CNS glial cells other than type-1 astrocytes. This invention offers also therapeutic agents appropriate for the method to cure spinal cord injuries.

[0011] The glial cells to be used in this invention are unrestricted in origin; autologous or allogeneic or xenogeneic CNS glial cells can be used. Among them autologous cells and allogeneic cells are preferable. For clinical application, autologous cells can be harvested from the injured spinal cord of the patients themselves. Allogeneic cells can be harvested from aborted embryos or from the corpse of a brain/cardiac ~~death~~ dead person. Xenogeneic cells can be harvested from pig, monkey, or ~~some~~ other mammals. Since the CNS is an immunologically privileged site, transplanted xenogeneic cells can survive when a small amount of immunosuppressant drugs is administered. ~~Resource of the~~ CNS glial cells ~~is~~ are preferably obtained from embryos, neonates or young animals but can also be obtained from older ~~aged~~ animals. They can be harvested preferably but not necessarily from the spinal cord. Any other part of the CNS, for example, the cerebral cortex, brainstem or the whole brain can be ~~the source of supply~~ used to obtain glial cells. Glial cells can also be derived from embryonic stem cells or neural stem cells ~~can also be the source of supply~~. Neural

stem cells are classified into adult type, embryonic type, and neuroepithelial type. They can be harvested not only from embyos or neonates but also from adults. Glial cells can be prepared from these resources by conventional biotechnologies using the ~~stimulant of~~ stimulants EGF, bFGF, CNTF, retinoic acid or T3 [(Genes Dev., 10, 3129-3140 (1996), Neuron, 18, 81-93 (1997), J. Neurosci., 18, 3620-3629 (1998)).

[0012] Any cell culture technology can be used for the preparation of glial cells. For example, the spinal cord or the cerebral cortex that was extirpated aseptically is treated with proteinase (e.g., trypsin) to make single cells or a small cluster of cells. Subsequently they are seeded on a Petri dish and cultured in a ~~serum-contained~~ serum-containing medium for a certain period of time in a $CO_2$ incubator. During culture, neurons die early and mixed glial cells survive. For cell culture Dulbecco's MEM (DMEM) containing 10-20% of fetal bovine serum, F-10 medium, or RPMI1640 or some other media can be used. The medium ~~Medium~~ is exchanged every 3-4 days. ~~With~~ Within days astrocytes become dominant because of their vigorous proliferative potential. Oligodendrocytes can be increased by employing serum free medium when necessary. The percoll ~~Percoll~~ density gradient method or adhesive difference method is effective in separating oligodendrocytes from astrocytes.

[0013] The therapeutic agents ~~in this~~ of the invention can be prepared ~~appropriate~~ appropriately for application by making a cell suspension of the cultured glial cells in a culture medium or a suitable buffered solution like PBS. The ~~medium of~~ cell suspension media can contain medically permitted additives unless they depress activity of cells. Cell density for application can ~~be ranged~~ range from $10^3$-$10^6$ cells/μL, preferably $10^4$-$10^5$ cells/μL.

[0014] The method ~~to cure~~ of curing spinal cord injuries ~~is~~ entails injection of an effective amount of the suspension into the lesion site. The method is applicable for humans and other mammals, either for partial or complete transection. There is no restriction to the location of injury: any part of the medulla, cervical, thoracic, lumbar, or sacral segments can be involved. The method ~~is applicable for~~ can be applied to respiratory paralysis, quadriplegia, or paraplegia, irrespective of severity.

[0015] The method is ~~applicable~~ best applied to spinal cord injuries resulting from ~~by~~ a fall ~~accident~~ or from a sports accident, but is not necessarily limited to such traumatic accidents ~~accident~~. It may also be applicable, for example, to ~~a~~ cerebrovascular damage ~~to~~ of the pyramidal tract. The treatment can preferably be performed acutely, ~~namely~~ within 24 hours, and preferably within 8 hours of tissue damage. However, the treatment may also be performed in a chronic stage, for example, one week, 5 years, or even more than 10 years after injury. The latter is based on the findings that a considerable number of projection neurons survived 3 months after severance of axons (3 months in the rat correspond to more than 10 years in humans). It ~~appears,~~ therefore appears likely that regeneration of axons is possible provided local conditions in the lesion are ameliorated.

[0016] Any method can be employed to administer a suspension of the cultured glial cells to the lesion site ~~so far~~ as long as it is injected safely and unfailingly. For example, the spinal cord is exposed by laminectomy. ~~, then, the~~ The suspension can then be injected intramedullarly using a microsyringe under a surgical microscope. When high resolution MRI images are obtained the cell suspension can be injected without laminectomy as in the technique of lumbar puncture. The number of CNS glial

cells to be injected depends on the extent of injury. Usually it ranges from $10^3$-$10^7$ cells, preferably $10^5$-$10^7$ cells in total for an adult patient. Prior to the injection of cells, immunosuppressant drugs such as cyclosporins, tacrolimus (FK505), cyclophosamid, azathioprines, methotrexate, or mizoribin can be administered ~~administerd~~. It is indispensable when xenogeneic cells are transplanted.

[0019] Composition of the cultured mixed glial cells was ~~analysed~~ analyzed by antigen specific marker molecules and is listed in Table 1. The classification described in Neuroglia, Helmut Kettenmann et al., Oxford University Press (1995) was employed.

[0025] This invention provides a method for curing spinal cord injury by injection of cultured mixed CNS glial cells into the lesion. The invention achieved repairing of neural connections that, after treatment, were hardly distinguishable from normal in amount (the number of projection neurons), length (extension of axons), path and termination of neurons, extension of axons, path, and termination. Recovery occurs rapidly from complete paraplegia, as demonstrated by ~~to the extent of not only~~ weight support ~~but also~~ and walking with hind-forelimb coordination. This breakthrough has hitherto been unachieved despite various endeavors to cure spinal cord injury and provides strategies for effective therapy that ~~is~~ are absent at the moment. When effective therapy is developed, it will reduce the physical and mental burdens of the patients and their family caregiver, and save ~~a~~ the heavy burden of medical and social welfare costs.